510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:
k120326

B. Purpose for Submission:
New device

C. Measurand:
Creatine kinase isoform MB (CKMB)

D. Type of Test:
Quantitative fluorometric immunoassay

E. Applicant:
Radiometer Medical ApS

F. Proprietary and Established Names:
AQT90 FLEX CKMB Test Kit
AQT90 FLEX CKMB CAL Cartridge
AQT90 FLEX LQC Multi-CHECK, Levels 1-3

G. Regulatory Information:

Device Name	Product Code	Classification	Regulation	Panel
AQT90 FLEX CKMB Test	JHX: Creatine phosphokinase/creatine kinase or isoenzymes test system	Class II	21 CFR § 862.1215	Clinical Chemistry (75)
AQT90 FLEX CKMB CAL cartridge	JIT: Calibrator	Class II	21 CFR § 862.1150	Clinical Chemistry (75)
AQT90 FLEX LQC Multi CHECK, Levels 1-3	JJY: Quality control material	Class I, reserved	21 CFR § 862.1660	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
   See indications for use below.

2. Indication(s) for use:
   AQT90 FLEX CKMB Test is an *in vitro* diagnostic assay for the quantitative determination of creatine kinase isoform MB in EDTA or lithium-heparin whole blood or plasma specimens on the AQT90 FLEX analyzer in point of care and laboratory settings. It is intended for use as an aid in the diagnosis of myocardial infarction.

   AQT90 FLEX CKMB CAL cartridge is for *in vitro* diagnostic use for the calibration of the CKMB Test on the AQT90 FLEX analyzer by establishing points of reference to estimate CKMB values.

   AQT90 FLEX LQC Multi-CHECK, Levels 1-3, is for *in vitro* diagnostic use. For use with the AQT90 FLEX analyzer as a liquid quality control serum (LQC) to monitor the precision of laboratory testing procedures for the analytes listed on the specification insert.

3. Special conditions for use statement(s):
   For prescription use.
   For point-of-care use.

4. Special instrument requirements:
   AQT90FLEX analyzer.

I. Device Description:

CKMB assay and calibrator:
The AQT90 FLEX CKMB Test consists of ten Test cartridges and one Calibration cartridge. The Test cartridge contains test cups whereas the Calibration adjustment cartridge contains both test cups and calibration adjustments cups.

The components are listed below:

- Mouse monoclonal anti-CKMB capture and tracer antibodies, approximately 500 ng/cup.
- Bovine serum albumin. Bovine γ-globulin. Mouse IgG as blocker substance for heterophilic antibody interference.
- Tris-(hydroxymethyl)-aminomethane (TRIS) buffer
- Sodium azide < 0.1%.
- Calibrator Only: contains native purified CKMB (only in the eight CAL cups with added antigen), approximately 2 ng/cup.

Each human donor unit used to manufacture this calibrator was tested using FDA-accepted methods and found nonreactive for Hepatitis B Surface Antigen (HbsAg), antibody to Hepatitis C (HCV) and antibody to HIV-1/HIV-2.

Control Material: AQT90 FLEX LQC Multi-CHECK, Levels 1-3 is comprised of three levels of control material (7, 25 and 100 ng/mL) in human serum. Each human donor unit used to manufacture this control was tested using FDA-accepted methods and found nonreactive for Hepatitis B Surface Antigen (HbsAg), antibody to Hepatitis C (HCV) and antibody to HIV-1/HIV-2.

J. Substantial Equivalence Information:

Predicate Device Name	Predicate 510(k) Number
Vidas CKMB assay	k962549
Vidas CKMB calibrators	k962549
Bio-Rad Laboratories Liquichek Cardiac Markers Plus	k050537

Comparison with predicate:

CKMB Assay		
Item	Proposed Device AQT90 FLEX CKMB Test	Predicate Device VIDAS CKMB Immunoassay (k962549)
Intended Use	*in vitro* diagnostic assay for the quantitative determination of creatine kinase. It is intended for use as an aid in the diagnosis of myocardial infarction.	same
Matrix	Whole blood and plasma (EDTA, Li-heparin)	Serum and plasma (EDTA, heparin)
Principle	Quantitative time-resolved fluorimetric one-step sandwich immunoassay	Enzyme-linked fluorescent immunoassay.
Reportable Range	1.5 to 300ng/mL	0.8 to 300 ng/mL
Analyzer	AQT90 FLEX	Vidas Vitek

Calibrator		
Item	Proposed Device AQT90 FLEX CKMB CAL Cartridge	Predicate Device VIDAS CKMB Calibrators A-F (k962549)
Indications for Use	For calibration of the CKMB Test.	Same
Constituents	Sixteen calibration adjustment cups, which contain dried reagents including native purified CKMB.	Purified cardiac CKMB in Tris buffer with stabilizers
Calibration adjustment interval	Once per lot of AQT90 FLEX CKMB Test cartridges and as often as required.	Upon new assay reagent lot number
In-use stability	24 hours on-board	30 days at 2-8 °C
Storage temperature	2-8 °C	-10 °C

Control		
Item	Proposed Device AQT90 FLEX LQC Multi-CHECK, Levels 1-3	Predicate Device Liquichek Cardiac Markers Plus Control LT (k050537)
Indications for Use	For use as a liquid quality control serum (LQC) to monitor the precision of laboratory testing procedures for the analytes listed on the specification insert.	Same
Form	Liquid	Same
Matrix	Human serum based	Same
Levels	Three	Same

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods
CLSI EP6-A: Evaluation of Linearity of Quantitative Measurement Procedures
CLSI EP07-A2: Interference Testing in Clinical Chemistry
CLSI EP09-A2: Method Comparison and Bias Estimation Using Patient Samples *CLSI EP17-A*: Protocols for Determination of Limits of Detection and Limits of Quantitation
CLSI C28-A3: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory
CLSI I/LA30-A: Immunoassay Interference by Endogenous Antibodies
EN 13640: Stability Testing of In Vitro Diagnostic Reagents
ISO 14971: Medical Devices - Application of risk management to medical devices
ISO 15223-1: Medical Devices - Symbols to be used with medical device labels, labeling and information to be supplied

L. Test Principle:

The test format for the AQT90 FLEX is an immunoassay based on time-resolved fluorescence using an europium (Eu) chelate as the fluorescent label. The test receptacles (300 µL test cups) for each assay contain the biotinylated antibodies used for the capture of the analyte, a separating layer of glucosides, and the europium-chelate labeled antibodies used to trace the captured proteins. The sample or diluted sample is added to the test cup together with assay buffer. The cup is then incubated to allow formation of the immuno-complex and washed to remove unbound antibodies and sample materials. Finally, the cup is exposed to excitation light and after a delay, the emitted light is measured by single photon counting; this measurement cycle is repeated up to 3,300 times. The total count (the response) is then compared to a calibration curve to obtain a quantitative measurement of the analyte's concentration in the sample.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   The precision of the AQT90 Flex CKMB Test on the AQT90 FLEX analyzer was evaluated using whole blood and plasma samples based upon the CLSI EP5-A2 guideline.

   For plasma samples evaluations, five levels of frozen native or antigen spiked lithium–heparin plasma samples were tested in

duplicate in two separate runs for 20-25 days at three POC sites (one instrument, three test kit lots per site). The number of samples tested for each level ranged from 58 to 96. For whole blood samples evaluations, fresh native or antigen spiked lithium-heparin whole blood samples were tested in five replicates in five separate runs within three hours at four POC sites (one instrument, one test kit lot per site), total N=25.
The results are summarized in the following tables.

Li Heparin Plasma Precision Study Results

Site	Mean ng/mL	Within run SD	Within run %CV	Total SD	Total %CV
1	L1=2.3	0.10	4.4	0.16	7.0
	L2*=4.2	0.15	3.7	0.24	5.7
	L3=12	0.4	3.0	0.7	5.5
	L4=44	1.2	2.7	2.1	4.8
	L5=288	7.9	2.7	10.7	3.7
2	L1=2.3	0.12	5.2	0.18	7.7
	L2*=4.1	0.20	5.0	0.27	6.6
	L3=12	0.4	2.9	0.5	4.3
	L4=44	1.1	2.4	1.8	4.1
	L5=287	8.3	2.9	9.8	3.4
3	L1=2.4	0.21	8.7	0.22	9.1
	L2*=4.4	0.15	3.5	0.19	4.3
	L3=12	0.3	2.5	0.4	3.3
	L4=44	1.0	2.2	1.3	3.0
	L5=290	4.5	1.6	6.9	2.4

*native sample

Whole Blood Precision Study Results

Site	Mean (ng/mL)	N	Within run SD	Within run %CV	Total SD	Total %CV
1	L1= 1.9	25	10.11	6.0	0.11	6.0
	L2*= 4.8	25	0.26	5.4	0.28	5.9
	L3=18	25	0.4	2.1	0.4	2.4
	L4=57	25	1.0	1.7	1.0	1.7
2	L1=2.9	25	0.15	7.6	0.18	9.3
	L2*=4.2	25	0.18	4.3	0.20	4.7
	L3=16	25	0.6	3.7	0.6	3.7
	L4=51	25	1.4	2.7	1.7	3.3
3	L1=2.4	25	0.13	5.4	0.14	5.9
	L2*=3.9	25	0.15	3.8	0.15	3.8
	L3=51	25	0.5	3.1	0.5	3.1
4	L4=217	25	2.2	4.4	2.2	4.4
5	L5=237	25	8.4	3.5	8.4	3.5

*native sample

b. Linearity/assay reportable range:

A linearity study was performed using EDTA natural patient plasma spiked with CKMB antigen. The high concentration and low concentration samples were mixed together to create 10 intermediate dilution samples. All samples were tested in replicates of ten with samples ranging from 1.2 to 374-ng/mL.
The linear regression correlation between the expected values and the measured values is:

$y = 1.01x + 0.68$, $r^2 = 0.9$

An additional linearity study was conducted using EDTA natural patient whole blood spiked with CKMB antigen. The high concentration and low concentration samples were mixed together to create 10 intermediate dilution samples. All samples were tested in replicates of ten with samples ranging from 1.3 ng/mL to 404 ng/mL. The linear regression correlation between the expected values and the measured values is: $y = 0.99 x + 1.08$, $r^2 = 0.99$

The linearity data provided support the sponsor's claims that the reportable range of this assay is 1.5 to 300 ng/mL for both whole blood and plasma samples.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability and Value Assignment

The antigen material, ERM-AD455/IFCC material, has been used to establish the metrological traceability chain of the AQT90 FLEX CKMB assay.

The calibrator value is internally assigned by the manufacturer and the factory-defined calibration data is provided in the form of a barcode. This data is used as a lot-specific reference curve, which is adjusted to the correct instrument specific signal level by using a CAL Cartridge with the same lot number. The protocols for value assignment and acceptance criteria were reviewed and are acceptable.

The control value assignment for the three CKMB levels of the AQT90 FLEX LQC Multi-CHECK control material is performed by Radiometer. The value assignment is traceable to master standards. The value assignment for each Multi-CHECK level includes multiple reagent lots, instruments, test days, and measurement replicates per control level, for a total of 60 measurements per control level. The assigned analyte value and range are lot-specific and printed on the specification label. The specification label has a lot-specific barcode, containing the same information that is printed (analyte level, assigned value, range levels, lot number and expiry date). This information is assessable through the AQT90 FLEX Analyzer. The protocols for value assignment and acceptance criteria were reviewed and are acceptable.

Stability

AQT90 FLEX CKMB Test and Calibration Cartridges: The stability studies were performed on three product lots of test and calibration cartridges. Based on the results, the sponsor claims that test and calibration cartridges have a shelf stability of 8 months at 2 to 8 ^{O}C, while the on-board stability is 23 days at 32 ^{O}C for the test cartridge and 24 hours at 32 ^{O}C for the calibration cartridge. The protocols for stability and acceptance criteria were reviewed and are acceptable.

AQT90 FLEX LQC Multi-Check*:* Stability studies were performed on three lots. Based on the results, the sponsor claims a shelf stability of 12 months at -18 to -26 °C, while the on-board stability is 4 days unopened at 2 to 8 ^{O} C or 2 hours opened at room temperature. The protocols for stability and acceptance criteria were reviewed and are acceptable.

d. Detection limit:

The Limit of Blank (LoB) determination was based on 60 replicate measurements of AQT Assay Buffer on a single AQT90 FLEX instrument and one test kit lot number. The Limit of Blank as determined by the upper 95th percentile is 0.09ng/mL. LoB is 0.5 ng/mL.

Limit of Detection (LoD) determination was based on 20 replicate measurements of 10 low samples on 2 AQT90 FLEX instruments and 2 test kit lot numbers. The LoD determination was calculated by using the determined LoB value and pooled SD values from LoD samples. The sponsor claimed that LoD is 1.0 ng/mL for both EDTA plasma and EDTA whole blood.

Limit of Quantitation (LoQ) determination was based on an interassay precision of >20% for 30 replicate measurements of 10 low samples on two AQT90 FLEX instruments and 2 test kit lot numbers. The Limit of Quantitation was determined to be 0.57ng/mL and 0.62 ng/mL for EDTA plasma and EDTA whole blood samples, respectively. The sponsor claimed that LoQ is 1.0 ng/mL for both plasma and whole blood.

LoB, LoD and LoQ are summarized in the table below:

CKMB	Value ng/ml
Limit of blank (LoB)	0.5
Limit of detection (LoD)	1.0
Limit of quantitation (LoQ)	1.0

The sponsor's claimed measuring range is 1.5 to 300 ng/mL.

e. Analytical specificity:

Interference studies were designed according to the CLSI EP7-A guideline. Two levels of human plasma (CKMB approximately 13 and 300 ng/mL) were spiked with known interference substances (52 drugs and 13 endogenous compounds) and samples were analyzed in five replicates on the AQT90 FLEX analyzer. In addition, two levels of whole blood samples (13 and 300 ng/mL) were also tested with the 13 endogenous compounds. Non-significant interference, defined as

recovery within ±10% compared to reference without any interfering substance, was observed with the assay. Results are summarized in the table below for the endogenous interference substances (both plasma and whole blood yielded similar results). The tests for unconjugated bilirubin, conjugated bilirubin, biotin, cholesterol, creatinine, glucose, hemoglobin, human IgG, triglycerides and urea were performed with Lithium Heparin as the anticoagulant. All other tests were performed with K_2EDTA as anticoagulant. The interferent study results are summarized below:

Interferents	Highest concentration tested showing non-significant interference
Bilirubin, conjugated	27 mg/dL
Bilirubin, unconjugated	27 mg/dL
Biotin	3 µ/L
Cholesterol	500 mg/dL
Creatinine	5.0 mg/dL
Fibrinogen	1000 mg/dL
Glucose	1000 mg/dL
Hemoglobin	1000 mg/dL
Human albumin	10000 mg/dL
Human IgG	5300 mg/dL
Triglycerides (Intralipid)	1500 mg/dL
Urea	200 mg/dL

Results of the 52 drugs tested that have non-significant interference are listed in the labeling.

No hook effect was observed for concentrations up to 7600 ng/mL with either EDTA plasma or EDTA whole blood.

Cross reactivity with CKBB and CKMM were evaluated at two levels of CKMB concentrations (1 ng/mL and 15 ng/mL). Samples spiked with either 2500 ng/mL of CKBB or CKMM were used for the studies. Studies were carried out for both plasma and whole blood samples. All the cross-reactivity results were less than 0.1%.

Potential interference from human anti-mouse antibody (HAMA) and Rheumatoid factor (RF) was evaluated by comparing the results of seventeen human HAMA and/or RF

plasma samples spiked with CKMB to reference sample (HAMA/RF negative) containing the same amount of CKMB. No negative interference for the assay was observed, which was defined as recovery within ± 10% of human HAMA/RF positive samples compared to a reference sample (HAMA/RF negative). The highest concentration test for HAMA was 1825 ng/mL and for RF was 8530IU/mL. The sponsor claims no interference from human anti-mouse antibody (HAMA) and Rheumatoid factor (RF).

f. Assay cut-off:
Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Paired human whole blood and lithium-heparin plasma samples were collected at three hospitals (POC sites) and measurements were obtained using the AQT90 FLEX CKMB Test run on the AQT90 FLEX analyzer. Frozen plasma samples were sent to a central laboratory and comparative measurements were obtained on the predicate device (VIDAS CKMB assay). Nine samples were spiked to supplement the samples in the hard-to-find, upper range. Singlet set of results from the candidate device was used to compare against the mean results of the predicate device. The results were analyzed by Passing-Bablok regression. The correlation data is summarized below:

AQT Fresh Whole Blood vs. Vidas Frozen Plasma	POC 1	POC 2	POC 3
Number of samples	43	46	48
Range of data (ng/mL)	1.8-235	1.7-277	1.5-206
Slope	0.93	0.93	0.96
Slope 95% C.I.	0.87-0.99	0.85-1.00	0.89-1.05
Intercept (ng/mL)	-0.2	0.0	-0.5
Intercept 95% C.I.	-0.5-0.1	-0.4-0.3	-1.0- -0.2
r^2	0.99	0.98	0.95

AQT Fresh Plasma vs. Vidas Frozen Plasma	POC 1	POC 2	POC 3
Number of samples	44	46	48
Range of data (ng/mL)	2.1-238	1.7-279	1.9-218
Slope	0.94	0.96	0.93
Slope 95% C.I.	0.89-0.98	0.88-1.01	0.89-1.05
Intercept (ng/mL)	-0.3	0.0	-0.4
Intercept 95% C.I.	-0.5-0.1	-0.2-0.3	-1.0-0.0
r^2	0.99	0.99	0.95

b. matrix comparison:

Four matrix- anticoagulant combinations were evaluated in the study, which were Lithium-Heparin whole blood, Lithium-Heparin plasma, EDTA whole blood and EDTA plasma. The study followed CLSI guideline EP9-A2, Method Comparison and Bias Estimation Using Patient Samples. The results of plasma versus whole blood were analyzed by Passing-Bablok regression. Results are summarized below:

Matrix	Regression	r^2	n	Data range
Li-Hep Plasma vs. Li-Hep WB	y = 1.04x + 0.1	1.00	75	1.7-294
EDTA Plasma vs. EDTA WB	y = 1.03x + 0.0	1.00	74	1.9-288

Anticoagulant	Regression	r^2	n	Data range
EDTA WB vs. Li-Hep WB	y = 1.03x + 0.0	1.00	65	1.7-198
EDTA Plasma vs. Li-Hep Plasma	y = 1.02x + 0.0	1.00	71	1.9-197

The sponsor concluded that lithium heparin and EDTA are acceptable anti-coagulant and the four anticoagulant-matrix combinations can be used.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

None

5. Expected values/Reference range:

Whole blood (lithium-heparin and EDTA) and plasma (lithium-heparin and EDTA) were obtained from 691 apparently healthy individuals (ages: 21 to 91 years) from a geographically diverse U.S. adult population (388 women and 303 men) and analyzed using the AQT90 FLEX CKMB assay. The 97.5^{th} Percentile was determined to be 6.9 ng/mL for women and 11 ng/mL for men. It is recommended that each laboratory should establish its own reference range.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.